EXHIBIT 14

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Clinical Protocol

Phase I Study of Immunotherapy of Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions

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This protocol is identical in background, goals, patient monitoring, and drug preparation and supply as the master protocol "Phase I/II Trial of HLA-B7 Plasmid DNA/DMRIE/DOPE Complex as an Immunotherapeutic Agent in Solid Malignant tumors by Direct Gene Transfer." In addition, it contains the details of patient eligibility and treatment specific to a study of metastatic renal cell carcinoma proposed by and to be carried out at the University of Chicago.

SCHEMA

Prior to discussing protocol entry with the patient, call the Research nurse to insure that a place on the protocol is open to the patient.

Escalating doses will be evaluated. Groups of patients will receive either escalating doses of an intralesional injection of the test agent into one metastatic lesion in a single session (schedule A) or repeated injections at the same dose (schedule B).

SCHEDULE A

Group	*Number of patients*	*mcg of DNA*
A-1	3	10 mcg day 1 only
A-2	3	50 mcg day 1 only
A-3	3	250 mcg day 1 only

SCHEDULE B
(WILL BE INITIATED AFTER SAFETY OF SCHEDULE A AT 50 MCG HAS BEEN ESTABLISHED)

Group	*Number of patients*	*mcg of DNA*
B-1	3	10 mcg day 1 and 15
B-2	3	10 mcg day 1, 15 and 30

SCHEDULE A

A 1 to 3 REG	Rx (day 1)	4 wks Re-evaluation	PR—may be retreated once <PR—Observation

SCHEDULE B

B-1 REG	Rx (day 1)	Rx (day 15)		4 wks Re-evaluation	PR—cycle of 2 injections 15 days apart may be repeated once <PR—no Rx; Observation
B-2 REG	Rx (day 1)	Rx (day 15)	Rx (day 30)	2 wks Re-evaluation	PR—cycle of 3 injections each 15 days apart may be repeated once <PR—no Rx Observation

1.0 Background

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells, are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5–10% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor cells with high efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma anc colon adenocarcinoma are responsive to modulation of immune function, because the immune system can be induced to recognize tumor associated and tumor specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have given about a 10—20% response rate. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive transfer of cytotoxic T cells (CTL's), which have shown promise in animal models (1–6) and in man (7–10). More recently, molecular genetic interven-

tions have been designed in an attempt to improve the efficacy of immunotherapy.

Nabel and colleagues at the University of Michigan are investigating a novel molecular genetic intervention for human malignancy that enhances the immune reponse to tumors by in vivo gene transfer. This immunotherapeutic approach based on animal model work (11, 12) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7, introduced into human tumors in vivo by DNA/lipid complex transfection. The direct intratumoral injection approach is used. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells. The introduction of an allogeneic MHC gene directly into tumors in vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens.

In a preliminary trial in humans with malignant melanoma Nabel treated 5 patients with malignant melanoma. Three patients received 3 treatments, totaling 0.87 μg of DNA intratumorally, and 3 patients (2 additional patients plus one of the original 3 patients) received cumulative dose of 2.58 μg of DNA via three treatments. No toxicity resulted from this form of treatment and there was no formation of anti-DNA antibody or autoantibody. There was no plasmid DNA detectable in the blood by PCR following gene transfer (tested on days 3–7 post transfection at ~2 pg/ml sensitivity).

Evidence of gene transfer was found on biopsy of the injected tumor. The biopsy samples were analyzed for plasmid DNA, mRNA coding for HLA-B7 and the expression of HLA-B7 protein. In 4 of the 5 patients, plasmid DNA and HLA-B7 mRNA were detected within the treated nodules by PCR. HLA-B7 expression was confirmed in all treated nodules by immunohistochemical staining with a monoclonal antibody to the gene product. Two patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells. One of the 5 patients had a partial remission which involved cutaneous and visceral disease. (Nabel, et al., PNAS, in press).

These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7 , but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

Several improvements that may increase the convenience, safety and efficacy of the procedure have been introduced since the original Nabel studies including:

- an improved cationic lipid formulation, DMRIE/DPOE*;
- DNA plasmid construction to optimize expression

The efficacy of transfection was improved for the following reasons. Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner (Vical) in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency in vitro compared to the formulation previously used by Nabel, et al. More importantly, this formulation does not aggregate at high concentrations. This characteristic thus allows higher absolute concentrations of DNA and lipid complex to be introduced into experimental animals without toxicity. Because of these properties, it now becomes possible to introduce 100–1000 times more DNA which could allow the study of an expanded dose response gene expression in vivo.

The vector improvements are divided into two categories for this proposal. In the first case expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the β-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface. This is important because some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. It has been found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. These modifications have been incorporated in the study drug to be used in this submission. The study drug is identical to the study drug fully characterized in Dr. Nabel's RAC submission of June 7, 1993, which was unanimously allowed. This study will investigate the administration of the study drug in metastatic renal cell center.

1.2 Background

1.2.1 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery in vivo provided a model system for the introduction of recombinant gene-specific sites in vivo. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (13,14). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA lipid complex (12). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against

*DMRIE, 1,2-dimytistyloxypropyl-3-dimethyl-hydroxyethyl ammonium; DOPE, dioleoyl phosphatidylethanolamine bromide.

the HLA-B7 molecule was persistant. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site in vivo. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene in vivo (12).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product in vivo. In addition, this approach provided a general method for the induction of a specific site in vivo. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy was developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (11). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.2.2 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (15,16) and lymphokine-activated killer (LAK) cells (17). Cytolytic T cells which infiltrate tumors have been isolated and characterized (18). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (3,19). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (20,21), or by activation induced by substances such as BCG (22).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-α (TNF-α) (23) and lymphotoxin (24) can result in neoplastic cell death. Interferon-γ (IFN-γ) markedly increases class I MHC cell surface expression (25,26) and synergizes with TNF-α in producing this effect (27). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (28), and interleukin-2 (IL-2) activates Leu-19$^+$ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (17,29,30). The LAK cells lyse tumor cells without preimmunization or MHC restriction (31). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (32).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanisms to escape host defenses, perhaps through evolution of successively less immunogenic clones (33). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (34–38). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (39–43). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (40,41). The absolute level of class I MHC expression, however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (44).

The immune responsed to tumor cells can be stimulated by systemic administration of IL-2 (45), or IL-2 with LAK cells (46,47). Clinical trials using tumor infiltrating lymphocytes are also in progress (48). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (49). Expression of IL-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (50). These studies demonstrate that cytokines, expressed at high local concentration, are effective anti-tumor agents.

Nabel and co-workers have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (51–55), or expression of allogeneic antigens introduced by somatic cell hybridization (56,57). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection in vitro. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene (H-2L^d) into an H-2^b tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (58). In another instance, transfection of H-2^b melanoma cells with the H-2D^d gene did not lead to rejection (59), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (60). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (61). Several subclones which were selected in vitro and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggest that clone-to-clone variation in in vivo growth and tumorigenic capacity may result in other modifications of cells caused by transfection of the subcloning procedure, which affects their tumorgenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly in vivo.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in out animal model studies designed to enhance the immunogenicity of tumors in vivo. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduciton of genes into tumors by in vivo infection using retroviral vectors or by DNA lipid complex mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection in vivo.

1.2.3 Intra-Lesional Therapy

The Department of Diagnostic Radiology at the University of Chicago has extensive experience in CT and sonographically-guided needle biopsy. Sonographically-guided biopsy of metastatic lesions can be carried out with a high degree of accuracy and safety. It is almost always possible to obtain a core rather than an aspirate of tissue by using a 16–19 gauge needle, needles with cutting ends, and improved biopsy sampling technology. In a review from the Mayo Clinic, overall accuracy was 91% in the biopsy of 126 consecutive masses of various histologic types 3 cm or less in diameter. It is almost always possible to obtain a core rather than an aspirate of tissue by using a 16–19 gauge needle, needles with cutting ends, and improved biopsy sampling technology. In a study from the Mayo Clinic of 1,000 consecutive CT-guided biopsies, the rate of complication from the use of an 18-gauge biopsy needle was 0.3%, the same as the rate of complication from the use of a 21-gauge needle. This capability allows, in one sitting, biopsy of the intended target for gene transfer, documentation of metastatic cnacer in the lesion, and delivery of the DNA/liposomal mixture to the targeted nodule(s). For purposes of this protocol, nodules at least 2 cm in size that are easily accessible will be selected to further ensure successful target injection and to reduce the possibility of morbidity.

One advantageous feature of sonographically-guided delivery of DNA/liposomal complex is that the distribution of the fluid within the injected area can be visualized and documented on videotape. Apparently, the release of small bubbles from dissolved air in fluids at room temperature injected into tissue causes the area perfused to become hyperechoic. This allows the radiologist to observe the regions of the nodule being injected.

Excellent facilities exist at the University of Chicago for carrying out sonographically-directed biopsy and delivery of materials at deep sites in the body. For this in vivo gene transfer study, patients will be admitted to the Hematology/Oncology service at the University of Chicago Hospital. Invasive procedures in the ultrasound suite are carried out under conditions in which a general surgeon is immediately available in the extremely rare instance of the acute complication of bleeding. An operating room is always immediately available for such circumstances. A CT scanner will also be available to visualize localized bleeding. Following the procedure, the patient will be kept in the diagnostic radiology area until stable. The patient will then be transferred to the inpatient Hematology/Oncology ward.

The chemotherapy, pharmacy, and pharmacology shared resources of the University of Chicago Cancer Center are available and will support this project. The pharmacy will assure the storage, preparation, and delivery of the final DNA/liposomal product to the ultrasound suite when needed.

The design of this trial is similar to standard phase I clinical trials. HLA-B7 negative patients with metastatic renal cell carcinoma will be enrolled. Patients must have at least 2 metastatic lesions or one metastatic lesion and an intact primary which are clearly measurable by CT scan and one of which is appropriate for sonographically-guided biopsy and injection. Patients will be treated on one of two schedules. On schedule A, the recombinant DNA will be administered in escalating doses to groups of 3 patients at each of 3 dose levels: 10, 50, and 250 mcg. On schedule A, each patient will receive only a single injection. Each of 3 patiens will be observed for toxicity and response for 30 days before patients will receive DNA at the next planned higher dose, 50 mcg. The same procedure will be followed at this dose level before proceeding to the final dose level, 250 mcg. Schedule B will be initiated in new patients after all 3 patients given 50 mcg DNA have been observed and found not to have unacceptable toxicity. On schedule B-1, a group of 3 patients will receive 10 mcg DNA days 1 and 15. If at 30 days no patient has unacceptable toxicity, a second group of 3 new patients, group B-2, will receive 10 mcg DNA on days 1, 15, and 30.

2.0 Goals

2.1 To determine safety and toxicity of direct intralesional injection of increasing amounts of a DNA/lipid mixture: VCL-1005 (HLA-B7/DMRIE/DOPE) into tumors in selected patients with metastatic renal cell carcinoma. Escalating treatment regimens will be used and tumor growth evaluated.

2.2 To measure the cytotoxic T-cell activity directed towards antigens on tumor cells other than HLA-B7.

2.3 To measure humoral and cellular immune responses to HLA-B7.

2.4 To confirm expression in vivo of the HLA-B7 gene in the tumor cells.

2.5 To characterize the clinical response to escalating doses of the study drug by assessing the size of the injected tumor and of other tumor masses that may be present.

3.0 Patient Eligibility

3.1 Inclusion criteria

Prior to discussing protocol entry with the patient, call the Research nurse (Diana Karius, R.N., M.S.N., 702-2085 or pager 6732) to insure that a place on the protocol is open to the patient.

3.1.1 Histologically-confirmed metastatic renal cell carcinoma.

3.1.2 At least two metastatic lesions or one metastatic lesion and an intact primary that are clearly measurable in two dimensions on CT scan. One metastasis must measure at least 2.0 cm in greatest diameter and be accessible for intralesional injection.

3.1.3 Patients must have had prior standard therapy for their disease and have become unresponsive to it or have made the decision that other therapy would not be of any benefit.

3.1.4 Must be ≥18 years old.

3.1.5 The following laboratory values within 15 days of registration:

white blood count > 3,000.

platelet count > 100,000.

Hemoglobin > 9 g/dl.

prothrombin time < 1.5 × control.

serum creatinine ≤ 2.0 mg/dL.

total bilirubin ≤ 1.5 unless due to Gilbert's disease.

SGOT, SGPT < 3× the upper limit of normal unless due to disease.

3.1.6 Karnofsky Performance Scale (KPS) score ≥ 70.

3.1.7 Estimated life expectancy of at least 12 weeks.

3.1.8 HLA typing must indicate that the patient is HLA-B7 negative.

3.1.9 Must be anti-HIV antibody negative.

3.1.10 Women/men of reproductive potential agree to use effective contraception.

3.1.11 Patients must be immunocompetent by antigen skin test.

2.1 Exclusion Criteria

3.2.1 Positive antibody to HIV.

3.2.2 Active autoimmune disease.

3.2.3 Hepatitis (acute or chronic active).

3.2.4 Active infection.

3.2.5 No ratiation, biologic therapy or chemotherapy with 4 weeks (6 weeks for mitomycin or nitrosourea).

3.2.6 No corticosteroids within 2 weeks.

3.2.7 Diabetes mellitus not controlled by medical treatment.

3.2.8 Psychiatric illness that may make compliance to the clinical protocol unmanageable or may compromise the patient's ability to give informed consent.

4.0 Test Schedule

Tests and procedures	*<15 Days prior to registration*	*During course of Rx days 3, 15, and 29[1]*	*Follow-up: at 8 wks post Rx and q 8 wks until PROG, then q 8–12 wks*
History and exam, tumor meas., ht, wt, KPS	X	X	X[9]
Vital signs (pulse, blood pressure, temperature)	X	X[1]	
WBC, Hgb, PLT	X	X	X
Chem-17, SGPT	X	X	X
PT, PTT	X	Days 15 and 29 only	Day 57[6]
EKG	X		
Chest x-ray	X		
CT scan and/or ultrasound	X[7]	Days 15 and 29 only	X
Tumor biopsy, guided by ultrasound	X	Days 15 and 29 only	Days 43[6] and 57[6]
Urinalysis	X		
HBAg, HIV titer	X		
HLA-B7 phenotype	X[8]		
Assay for cytoxic T-cells and antibody[2]		X	X
Peripheral blood sample	X[3]	X[4]	Day 57[6]
Antinuclear antibodies	X	Day 29 only	Day 57[6]
Serum preg. test[5]	X		

1. Prior to each biopsy and treatment and then every 15 minutes until stable.
2. Biopsy tissue will be assayed for the presence of the HLA-B7 gene by PCR. Expression of the HLA-B7 gene will be looked for immunohistochemically. When sufficient materials are present, attempts to assess the development of cytolytic T-cell response to the cells of the tumor will be evaluated. Tissue will be "snap" frozen in liquid nitrogen (LN) and sent in LN to a central lab facility (The Arizona Cancer Center).
3. 45 ml of clotted blood for serum storage and 45 ml of anticoagulated blood for mononuclear cell cryopreservation will be obtained and sent to a central lab facility (The Arizona Cancer Center).
4. 20 ml of clotted blood will be obtained to look for development of antibodies, and 20 ml of anticoagulated blood for mononuclear cell cryopreservation will be obtained and sent to a central lab facility (The Arizona Cancer Center).
5. For women of childbearing potential only. Must be done within 7 days prior to registration.
6. Days 29, 43, and 57 are approximate. The goal will be to obtain the studies as close as possible to 4, 6, and 8 weeks +/− 2 days.
7. Baseline CT scan must be done within 4 weeks of treatment.
8. At any time prior to treatment.
9. Survival follow-up will be life-long.

5.0 Stratification Factors

None.

6.0 Registration/Randomization Procedure

6.1 Prior to discussing protocol entry with the patient, call the Research nurse to insure that a place on the protocol is open to the patient.

6.2 To register a patient, call Scott McKay (702-2084) in the Data management office.

6.3 A signed consent form must be on file in the data management office before a patient may be registered.

6.4 Patient eligibility and the existence of a signed consent form will be checked by data management personnel before a patient will be registered into this study. Dose level will be given.

6.5 Treatment on this protocol must be given at the University of Chicago on an inpatient basis under the supervision of an attending physician in Hematology/Oncology. Actual intralesional injection of tumor will be carried out by Dr. Gary Sudakoff or one of his associates in the Department of Diagnostic Radiology.

6.6 Treatment must begin within 15 days of registration.

6.7 Pre treatment tests must be completed within the guidelines specified on the test schedule.

7.0 Drug Information/Protocol Treatment

7.1 The study drug (VCL-1005) will be supplied by Vical as two sterile vials containing (i) HLA-B7 plasmid DNA, and (ii) DMRIE/DOPE lipid mixture. Diluent will be lactated Ringer's. All components will be stable for at least eight weeks under recommended storage conditions (DNA −20°C, DMRIE/DOPE 4°C). The materials will be supplied by Vical Inc.

This study drug is composed of plasmid DNA coding for the complete human MHC HLA-B7 formulated with the cationic lipid mixture DMRIE/DOPE (lipid complex formulation). The DNA concentration is 1.0 mg/ml (see Investigator's brochure for complete details of product characteristics and preparation).

Lactated Ringer's is readily available at the site.

7.2 Identification and localization of a specific metastasis for injection using ultrasound (or CT scan if not visible by ultrasound) meeting the criteria stated in section 3.0 will be carried out by Dr. Gary Sudakoff, or one of his associates, prior to admission. Tumor lesions will be selected for treatment if they are accessible to intratumor administration by direct needle injection. These metastatic lesions will be located at any accessible site such as skin, lymph nodes, lung, liver, kidney or spleen for renal cell carcinoma. If necessary, the study drug will be injected with the aid of sonographic visualization of the metastasis. In all phases of the study, a tumor nodule may be injected in multiple locations within a single tumor nodule. This procedures is intended to provide for multiple points of delivery of the product into the tumor mass, thereby maximizing the contact between the product and cells or the tumor, and in effect, bathing the tumor cells.

Once all pretherapy tests and procedures are completed, patients will be admitted to the Hematology/Oncology service at the University of Chicago prior to each intralesional injection.

7.3 DNA/lipid complexes are prepared immediately prior to administration. DNA is supplied in 1.0 mg/ml concentration in 400 µl lactated Ringer's solution. Lipid (DMRIE/DOPE) is supplied as a dried film. Each vial contains 77 µg DMRIE and 90 µg DOPE. Each vial is reconstituted with 400 µl lactated Ringer's solutions by vortexing until homogeneous. The contents of the lipid vial is transferred into the DNA vial and mixed well by repeated inversion. The final concentration of the HLA-B7 plasmid DNA is 500 µg/ml. The amount of DNA injected into each tumor will range from 10 µg to 250 µg. Lower doses, 10 and 50 µg, will be prepared in a similar fashion or formulated as dilutions with lactated Ringer's.

7.4 The appropriate volume of DNA/lipid mixture prepared in the University of Chicago Pharmacy will be injected into the pre-selected metastasis on day 1. The injection is done by the diagnostic radiologist using standard procedures in the Diagnostic Radiology Suite. The needle will be inserted under direct sonographic (or CT) visualization. A variable volume of lipid preparation (up to 4 ml) will be used but all DNA concentrations will be kept constant per dose level.

Prior to injection and following placement of the needle, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Up to 4 ml of the DNA/lipid mixture will be injected as described above. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm Hg, the injection will be terminated immediately, and the patient will be closely monitored until blood pressure has normalized.

Immediately after the injection, a blood sample will be obtained to check serum enzymes, blood chemistries and cell counts, and to analyze by PCR for the presence of HLA-B7 Plasmid DNA in the peripheral blood. Every patient will be observed for 23 hours and another blood collection will be drawn. If there are no complications, the patient will be closely observed. All toxicities will be graded according to the WHO recommendations (see Appendix 2).

7.5 Amounts of DNA to be injected:

SCHEDULE A

	Number of patients	*mcg of DNA*
A-1	3	10 mcg day 1 only
A-2	3	50 mcg day 1 only
A-3	3	250 mcg day 1 only

SCHEDULE B
(WILL BE INITIATED AFTER SAFETY OF SCHEDULE A AT 50 MCG HAS BEEN ESTABLISHED)

	Number of patients	*mcg of DNA*
B-1	3	10 mcg day 1 and 15
B-2	3	10 mcg day 1, 15 and 30

7.6 Criteria for re-treatment: Patients with a partial response at 4 weeks after the last injection of their initial course may receive an additional course of treatment identical to the first treatment, i.e., on Schedule A, at 4 weeks a responding patient may receive a second injection at the same dose initially given; on Schedule B, 4 weeks after two injections (B-1) and 4 weeks after three injections (B-2), responding patients may receive a second course identical to their first course.

7.7 The primary objectives of this investigation are to determine the feasibility and safety of intralesional injections of DNA/lipids into patients with renal cell carcinoma. Patients will be entered in groups of 3 at each dose level and observed for at least 4 weeks before entry of additional patients.

Number of patients with grade 3 toxicity (World Health Organization [WHO])	*Instructions for entry of additional patients*
0	Escalate to next dose level
1	Enter 3 more patients at same dose level
2 or 3	Discontinue further patient entry at that level and enter 3 more patients at the previous level

7.8 The *maximum tolerated dose* will be defined as the highest dose producing grade 2 toxicities in < 2 of 6 patients. It is possible for the maximum tolerated dose to be different for Schedula A and Schedule B.

8.0 Treatment Modification Based on Toxicity

Toxicity	*Dose reduction*
(For liver metastases only) SGOT, SGPT or alk phos >3× baseline	50%
Direct bili 0.2 mg/dl above ULN	50%
Other Rx complications or medical conditions with substantial risk to patient	No further Rx
Symptomatic tumor progression requiring other Rx e.g. RT or chemo	No further Rx

9.0 Ancillary Treatment

Symptomatic care may be given as required with medications such as antiemetics and analgesics. However, administration of corticosteroids will require that the patient be removed from study.

10.0 Toxicity Monitoring and Adverse Reaction Reporting

Patients will be monitored and questioned at every visit (see Schedule of Events) regarding the occurrence and nature of any adverse experiences. An event is any change in the physiological or psychological state other than the primary condition that qualifies the patient for this study.

The Investigator must report to Mr. Steven Kradjian (ph 619-453-9777, available 24 hours.) upon occurrence of any life-threatening events (Grade IV) that may be attributable to administration of the study drug, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 3 working days to:

Steven A. Kradjian,
Director, Regulatory Affairs
Vical, Inc.
9379 Towne Centre Dr., Suite 100
San Diego, CA 92121

Patients will be taken off study immediately if:

Unacceptable toxicity (Grade III or IV) develops and is not easily corrected (refer to WHO toxicity sheet).

Development of Progressive Disease (see definition) requiring the institution of alternative treatments such as radiation, surgery or other drug therapy. If the Investigator believes that the patient's best interest requires a change of therapy.

At the patient's request.

10.1 Toxicities to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per WHO Criteria;

Toxicity/symptoms	*Baseline*	*Each evaluation*
Fatigue	X	X
Weight loss/anorexia	X	X
Nausea	X	X
Vomiting	X	X
Hemorrhage		X
Infection		X
Liver (SGOT, alk phos, and total bilirubin)	X	X

11.0 Treatment Evaluation

11.1 Patients will be evaluated in the Hematology/Oncology clinic at the University of Chicago at least every 4 weeks while receiving treatment, every 8 weeks until there is evidence of tumor progression, and then 8-12 week intervals thereafter as long as the patient is able to return to the University of Chicago.

11.2 Evaluation of tumor regression is a secondary endpoint in this clinical trial. The following criteria will apply to measurable indicator lesions:

11.3 Minimum size requirements for measurable indicator lesions:

11.3.1 Tumor masses with clearly defined bidimentional measurements. The minimum size of the indicator lesion depends on the method of measurement as follows:

Method of measurement	*Minimum size of largest tumor diameter*
Physical examination or chest x-ray (discrete lesion that can be measured with calipers)	1.0 cm
CT scan*	3.0 cm

Note: Special attention should be paid to identify the specific lesion being measured, and to define an anatomic landmark to identify the level of the cross-sectional image to facilitate serial measurements. Lesions 2.0–3.0 cm in diamteter may be used as indicator lesions if serial images are obtained at 0.5 cm intervals through the tumor.

11.4 Criteria for a Partial Response (PR)

11.4.1 Bidimensional indicator lesion(s).
50% reduction in the sum of the products of the largest perpendicular diameters of the indicator lesion(s), single or multiple sites, chosen prior to therapy. Response status will be determined separately for the injected tumor nodule and other measurable lesions that are not injected.

11.5 Criteria for Complete Response (CR)

Total disappearance of all evidence of tumor.
For a patient to qualify for complete response or partial response, none of the factors constituting progression may be present (see below).

11.6 Criteria for Progressive Disease (PD)

Tumor progression will be declared if one or more of the following criteria are met.

11.6.1 Appearance of new lesion(s).

11.6.2 Increase in tumor size.

11.6.2.1 Patients with measurable indicator lesion(s) who have met the criteria for partial response.
Fifty percent increase in the size of indicator lesion(s) compared to the *smallest* measurements while on study.

11.6.2.2 Patients with measurable indicator lesion(s) who have met the criteria for complete response.
Progression will be declared if there is reappearance of any tumor

11.6.2.3 Any new lesions.

11.6.3 Significant clinical deterioration that cannot be attributed to treatment or other medical conditions.

11.6.3.1 Weight loss > 5% body weight.

11.6.3.2 Worsening of tumor-related symptoms.

11.6.3.3 Decline in performance status >1 level on ECOG scale.

11.7 Criteria for stable (SD)

Failure to meet the criteria for complete response, partial response, regression, or progression.

12.0 Descriptive Factors

None

13.0 Treatment/Follow-Up Decision at Evaluation of Patient

Patients meeting the criteria for partial repsonse at 4 weeks after their single injection on Schedule A or after their last injection on Schedule B may be retreated once.

14.0 Pharmacologic/Immunologic Studies

14.1 Immunochemical staining will be done at a central laboratory facility (The Arizona Cancer Center) as specified by VICAL, Inc. Pre- and post-treatment tumor cells are stained with anti-HLA-B7 antibodies, ME-1, BB7.1, and GSP5.3 (G. Nabel, personal communication) to look for expression.

14.2 Presence of DNA from the HLA-B7 gene will be assessed by PCR amplification of cells obtained by biopsy of the treated site on days 8, 15, and 29 after injection of DNA/lipid complex. Genomic DNA is isolated by standard methods and a portion of the HLA-B7 gene is amplified and sequenced. Several primer sequences may be used (G. Nabel, personal communication). These studies will be carried out in a central laboratory facility.

14.3 Development of circulating antibodies to HLA-B7 will be evaluated. Autologous peripheral blood B lymphocytes will be EBV immortalized and subjected to in vitro gene transfer with the DNA/lipid complex. These autologous cells expressing the HLA-B7 gene will be used to assess the specificity of antibody response to the in vivo transfer of the gene. Evidence of cytolytic T-cells will be assessed if a sufficient amount of material is available for successful expansion of infiltrating T-cell population from cells in biopsy of the metastasis. These studies will be carried out in a central laboratory facility.

15.0 Statistical Consideration and Methodology

Descriptive statistics only will be performed due to the small number of patients.

16.0 Pathology Considerations

16.1 Patients entering this trial will already have had histologically-documented metastatic renal cell carcinoma. A biopsy is carried out as part of this protocol including the initial one to demonstrate unequivocally by histologic examination of a frozen section that the metastasis selected for injection of the DNA/lipid complex is, in fact, metastatic cancer. Biopsy samples are never to be place in fixative.

16.2 For biopsies in which a core of tumor has been obtained, the sample will be subdivided and at least half frozen in liquid nitrogen. The remainder may be subjected to tissue disruption in an attempt to obtain and expand T-cells present in the tissue.

17.0 Records and Data Collection Procedures

17.1 University of Chicago patient records.

17.1.1 The University of Chicago medical records will be utilized for patients. Data will be entered into a

data management file within 3 weeks after each evaluation of the patient. After the patient goes off treatment, follow-up information will be collected and entered into the data management file every 3 months.

17.1.2 Pathologic diagnosis made by frozen section is reported in a conventional way with a record being placed in the patient's permanent medical record and data management file.

17.1.3 Data from the central laboratory will be entered into the data management file. Summary reports of samples received will be sent to the principal investigator monthly.

17.2 Vical records

Case report forms, regulatory documents and study source documents will be collected and reviewed in conformance with FDA Good Clinical Practices.

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UNIVERSITY OF CHICAGO PROTOCOL #7079
Phase I Study of Immunotherapy of Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions

AMENDMENTS

Section 3.1.2:

1. There will be no upper limit size restriction for the metastatic lesion or primary renal tumor that is injected with the DNA/lipid complex. The lower limit size restriction will remain as stated in this section.

Section 4.0

1. Clinical follow-up and safety evaluation during the first 15 days of the protocol will be conducted on a protocol and patient specific basis, but will include blood collections on day 1 (prior to injection), day 8 and day 15. All patients will also be seen and examined by a University of Chicago Physician at these times. This pattern is to be repeated for subsequent doses in the multiple injection treatment group. It is believed that some follow-up symptoms and blood chemistries may be related to the the injections and not related to the DNA/lipid complex. Follow-up safety evaluations should be based upon clinical observations and patient feedback. However, the clinical chemistry and other indicators of product related events should be performed within 10 days of treatment.
2. The sample collection schedule to be shipped to the Arizona Cancer Center (the central lab facility) is as follows: Screening (days −14 to −1), days 1, 15, 29, week 6 and week 9 ± 1 week
3. The biopsy requirements are as follows: Minimum of 1 core (1–2 cm × 1 mm) snap frozen in liquid nitrogen; or 2 cores, one on wet ice, one snap frozen in liquid nitrogen; or 3 cores, one on wet ice, two snap frozen in liquid nitrogen
4. Biopsy with 18–20 gauge needle is recommended. To demonstrate transfection, biopsies should be taken from the injected tumor, as close to the injection site as possible.
5. Screening HLA-B7 phenotype will be performed at the Arizona Cancer Center. A 10 ml plasma sample should be shipped on wet ice in a Na heparinized tube.
6. All serum collections for the Arizona Cancer Center should be in a green top vacutainer with Na heparin. Shipment of samples from the University of Chicago should only take place on Monday, Tuesday or Wednesday. Shipment on Monday is preferred.

Section 7.4

1. Dose volume of the DNA/lipid complex administered may be increased up to 4 ml, according to the size of the tumor injected. In most cases, the pharmacy will prepare the product in a syringe of 3 ml Lactated Ringers. Following the injection of the product into the tumor, a 1 ml syringe of Lactated Ringers will be flushed through the needle to clear the needle and hub of any product.

THE UNIVERSITY OF CHICAGO
DIVISION OF BIOLOGICAL SCIENCES
Consent By Subject For Participation in Research Protocol

Protocol Number: 7079
Title of Protocol: Phase I Study of Immunotherapy for Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions

Patient Name: _____

Doctor(s) Directing Research: Nicholas J. Vogelzang, M.D. (312-702-6743)
Timothy M. Lestingi, M.D. (312-753-3636)
Gary Sudakoff, M.D. (312-702-2562)

You are being asked to participate in a research study. The doctors at the University of Chicago Hospitals and the Division of The Biological Sciences study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent. This consent form gives detailed information about the research study which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

I. NATURE AND DURATION OF PROCEDURE(S):

As you are aware, your kidney cancer cannot be cured by medicine, surgery, or radiation. In this study, a procedure will be performed that may help to fight this disease in future patients. Because the procedure is experimental, it is possible that you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new product which will attempt to induce tumor regression in patients with widespread kidney cancer. Because this is a new, experimental procedure, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this procedure on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein—known as HLA-B7—causes the cells which contain it to be recognized as "a foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this procedure will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of procedure which stimulates your lymphocytes is called immunotherapy.

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatinet. These tests include: 1) blood tests; 2) x-rays of the brain, chest, and abdomen; 3) a blood test for hepatitis; and 4) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV or hepatitis virus, you may not participate in this study. These initial blood tests require that about 45 ml (3 tablespoons) of blood be taken.

If you qualify for this study, we will inject the experimental mixture containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to four times. The time of treatment is usually ~30 minutes. The treatment may be repeated every two weeks for a total of three. Blood samples (about 90 ml or 6 tablespoons) will be obtained biweekly for up to the first three months. A CT scan will be performed before initiation of each treatment and at the end of the two month study period. Your blood lymphocytes will be tested for their ability to response to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocanine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to the product injection and at intervals of two weeks up to four times after the last injection.

You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors.

Follow-up

After you receive the product injection, you will be discharged from the hospital after a one night stay if you have no other significant medical problems. You will be required to return to the University of Chicago Medical Center for follow-up studies described above for at least eight weeks after the trial has begun. It is possible that we may ask you to return after this time if additional tests will be needed. We also will maintain life-long telephone contact with you or your doctor if you are not receiving care at the University of Chicago so that we may check on your progress. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects. It should be emphasized that the follow-up on this study will be life-long.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks of side effects encountered. In the single injection study group, patients will receive one injection and will be eligible for a second injection if there is a sufficient response from the first injection. In the multiple injection study group, patients will receive either 2 or 3 injections in the first month, and will be eligible to receive the same series of 2 or 3 injections for one additional month if there is sufficient response from the first series. Your physicians feel that the risks of your disease are much greater than the risks of treatment as outlined above. Futhermore, your physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy, or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

II. POTENTIAL RISKS AND BENEFITS:

Risks and Side Effects

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from the needle injections or tumor biopsies. You may have mild discomfort and bleeding from the tumor biopsy. You will be given a local anesthetic to minimize the discomfort. Second, even though the DNA inserted into your tumor is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could become abnormal after a long period of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treatment of any potential bacterial infections. Finally, there could be bruising at the site where you will be getting your blood drawn as well as possible inflammation or infection of the vein. Of course, care will be taken to avoid these complications.

We emphasize that this procedure, called direct gene transfer, has been used in only a few human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur. This new form of therapy may attract considerable attention from the media and scientific community; however, your identity will be protected from the media and from scientific publication.

No data exists regarding the effects of this therapy on human reproductive function. Therefore, you should use adequate contraception to avoid the pregnancy of you or your spouse during the course of this treatment.

III. ALTERNATIVE THERAPIES:

There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms. These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

IV. COSTS:

You will not be paid to take part in this study.

Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinical visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The costs of tests and treatments unrelated to this study will be handles as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.

CONSENT FOR AUTOPSY

To fully evaluate the effects and safety of gene therapy, it will be necessary to obtain as much information as possible. In the unlikely event or occurrence of your death, either during

this protocol or after its completion, evaluation of your organs will be a very valuable method to see the full effects of gene therapy. Therefore, if you participate in this study, we would like you to consider an autopsy in the event of your death. However, consent to an autopsy is not required to enroll on this trial.

AGREEMENT TO CONSENT

The research project and treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available at the University of Chicago Hospitals. Confidentiality of medical records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by applicable government agencies, including The Federal Food and Drug Administration, National Cancer Institute and the sponsor of the study, VICAL.

I understand that in the event of physical injury resulting from this research, The University of Chicago Hospitals will provide me with free emergency care, if such care is necessary. I also understand that if I wish, The University of Chicago Hospitals will provide non-emergency care, but the Medical Center assumes no responsibility to pay for such care or to provide me with financial compensation.

I, the undersigned, hereby consent to participate as a subject in the above described research project conducted at The University of Chicago Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have questions concerning my rights in connection with the research, I can contact the Institutional Review Board, at 312-702-1472.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor: _____ _____
Signature of Subject

Witness: _____ _____
Signature of Parent or Guardian
(if patient is a minor)

Date: _____ Time: _____ am/pm

I wish to undergo autopsy in the event of my death:

___ Yes ___ No

(If you answered "Yes" please also inform your next of kin.)
Revision date: 4/28/94